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## Distinctive Developmental Origins and Specificities of Murine CD5<sup>+</sup> B Cells

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### SUMMARY

CD5<sup>+</sup> B cells constitute a small fraction of cells in the spleen of adult mice that exhibit numerous features serving to distinguish them from the bulk of IgD<sup>+</sup> CD5<sup>-</sup> "conventional" B cells. In this review we focus on two major questions relating to this population: 1) the relationship of CD5<sup>+</sup> B cells to other B cells; and 2) the distinctive enrichment of particular autoreactive specificities in this subset. The nature of their origins is clarified by a thorough analysis of intermediate stages of early B-cell development in both fetal and adult tissues. The reactivity to bromelain-treated mouse red blood cells serves as a prototype system for the investigation of biased specificities in CD5<sup>+</sup> B cells. These lines of investigation lead us to propose that CD5<sup>+</sup> B cells in the adult are the remnant of a distinct fetal B-cell differentiation pathway wherein selection of cells from this fetal/neonatal population into the adult long-lived pool results in the overexpression of certain germline-encoded autoreactivities.

### INTRODUCTION

B cells in the periphery show considerable heterogeneity when analyzed for expression of IgM and IgD (Hardy et al. 1982). Most spleen B cells and essentially all lymph node B cells in normal healthy animals maintained under specific pathogen-free conditions fall in an IgM<sup>+</sup> IgD<sup>+</sup> fraction, but a minor fraction of IgM<sup>+</sup> IgD<sup>+</sup> cells is consistently found in spleen. Based on their similarity to the phenotype of B cells found in neonatal mice, these cells have often been regarded as "immature", recent migrants from the bone marrow. While such cells undoubtedly

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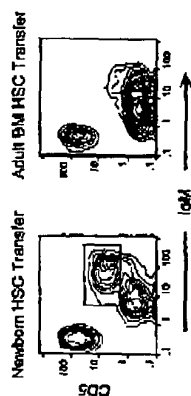


Figure 1. Differential repopulation of CD5<sup>+</sup> B cells using a hematopoietic stem cell-enriched fraction sorted from liver of newborn mice versus a comparable fraction sorted from bone marrow of adults. IgM<sup>+</sup> T1y-1<sup>+</sup> lineage cell fractions (0.2% of total) were isolated and injected i.v. into SCID mice irradiated (300R) the previous day. Two months later animals were sacrificed and cells washed out of the peritoneal cavity were stained and analyzed by flow cytometry with indicated reagents. Other tissues examined (thymus, bone marrow, spleen) were completely reconstituted with conventional B cells and T cells.

eventually become almost completely inactive in adult bone marrow. We sought to investigate this more completely by first clearly delineating the intermediate stages of early B-lineage differentiation in adult bone marrow and then comparing this to the process in fetal liver.

#### B-lineage differentiation in the bone marrow

Our approach has been to utilize flow cytometry multiparameter analysis using a set of reagents recognizing cell surface molecules differentially expressed during B-lineage development to discriminate intermediate stages in bone marrow (Hardy et al. 1991). Initially, we resolved B220<sup>+</sup> IgM<sup>+</sup> B-lineage cells in mouse bone marrow into four fractions based on differential cell surface expression of determinants recognized by 57 (anti-leukosialin, CD43; Bareuther et al. 1988, Guller et al. 1988), B1-1 (Wu et al. 1997), and 30T1 (anti-hepatocellular carcinoma antigen, HSA; Kay et al. 1990). Clear functional distinctions emerged by comparing these fractions: the largest fraction, lacking CD43, consisted of small cells that did not proliferate in Whitlock-Witte type stromal cell culture (Whitlock et al. 1987), whereas the CD43<sup>+</sup> fractions, provisionally termed Fr. A, Fr. B and Fr. C, could proliferate and differentiate in stromal culture (Hardy et al. 1991). Phenotypic alteration during such culture suggested an ordering of these stages from Fr. A to Fr. B to Fr. C and thence to CD43<sup>+</sup> small pre-B cells (see Fig. 2). Furthermore, functional analysis of sorted fractions demonstrated that the proliferation of these fractions changed from absolute contact dependence in Fr. A to contact independence in Fr. C (Fig. 3), consistent with progression from A to C since altered growth requirements after heavy chain rearrangement have been suggested previously (Sauter & Paige 1988). B220<sup>+</sup> CD43<sup>+</sup> cells in bone marrow can be divided into

cally do fall within this IgM/IgD fraction, other criteria and other surface markers can discriminate more mature cells with this phenotype, such as marginal zone B cells. One cell type with this distinctive IgM/IgD ratio is delineated by expression of CD5, constituting 1-2% of spleen.

It shows a variety of novel features that distinguish them from the bulk of IgD<sup>+</sup> CD5<sup>+</sup> "conventional" B cells (reviewed in Hayakawa & Hardy 1988, Kipps 1989, Kantor & Herzenberg 1993, Hardy & Hayakawa 1993). Among these are distinctive surface phenotype (IgM<sup>+</sup>/IgD<sup>+</sup>/B220<sup>+</sup>), novel aneuploid localization (enrichment in the peritoneal cavity), early appearance in ontogeny, secretion of certain autoreactive antibodies and increased frequency in several autoimmune mouse strains. Further work has demonstrated predisposition to unregulated growth or even lymphoma. The demonstration of a homologous population in human ("CD5<sup>+</sup> B cells") with many similar features, including early appearance and novel biases in specificity together with the long-recognized presence of CD5 on B-lineage chronic lymphocytic leukemia cells (Bourne et al. 1978) has stimulated interest in defining more precisely the relationship of this B cell subpopulation to the majority of conventional B cells.

In this review we focus on two issues: 1) defining the origins of CD5<sup>+</sup> B cells by a thorough analysis of intermediate stages of early B-cell development in both fetal and adult tissues; and 2) studying the expression of particular specificities and associated immunoglobulin V genes enriched in this population. We present the experimental background that supports our current understanding of CD5<sup>+</sup> B cells as the remnant of a distinct fetal B-cell differentiation pathway and suggest that the selection of cells from this fetal/neonatal population into the adult long-lived pool results in the over-expression of certain germline-encoded autoreactivities, such as antibody to bromelain-treated mouse red blood cells and intact thymocytes.

#### B-CELL DEVELOPMENT

Bone marrow is considered the primary site of B lymphopoiesis in adult mice. Surprisingly, several years ago we found that CD5<sup>+</sup> B cells were poorly generated from cell transfer of adult bone marrow, in contrast with good repopulation using early B-cell progenitor sources such as fetal or newborn liver (Hayakawa et al. 1985). More recently (Hardy & Hayakawa 1992), we compared SCID mice (that lack lymphocytes of their own; Bosma 1989, Hardy et al. 1989) reconstituted using fractions sorted to enrich for hematopoietic stem cells (Muller et al. 1986, Spangrude et al. 1986) and again found CD5<sup>+</sup> B cells when liver was used, but few when bone marrow was the source (Fig. 1). Our initial expectation was that two distinct sets of B cells were being generated in fetal liver, but that gradually one of these differentiation pathways or "lineages" diminished with age and

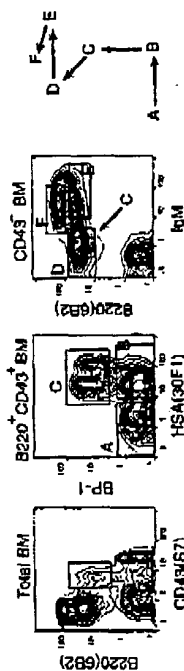


Figure 2. Diagram of B-cell differentiation in BM as resolved by correlated expression of B220(6B2), CD43(57), HSA(30F1), and IgM. Phenotypic transition *in vitro*, differences in functional activity and immunoglobulin gene rearrangement status all support the pathway diagrammed at the right hand side.

TABLE I  
Heavy chain rearrangement in B-lineage cells in bone marrow

Cell fraction	Fractional Retention of Germ-line	
	fragment 5' of D	fragment 5' of J
	mean (SE)	mean (SE)
Fr. A	1.05 (0.05)	0.95 (0.03)
Fr. B	0.98 (0.03)	0.39 (0.02)
Fr. C	1.00 (0.04)	0.17 (0.03)
Pre-B	0.50 (0.01)	0.05 (0.01)
B cell	0.50 (0.04)	0.05 (0.01)

three fractions using an alternate four-color separation with B220, CD43, IgM and IgD. Small pre-B cells (Fr. D) lack IgM and IgD, newly generated B cells (Fr. E) express only IgM and mature B cells (Fr. F) express both IgM and IgD.

Analysis of Abelson-transformed B-lineage cell lines has demonstrated an ordering of Ig recombination, first heavy chain D-J, followed by complete VDJ and finally light chain rearrangement (Yacobi et al. 1983, Alt et al. 1984). Therefore, it was important to characterize the status of immunoglobulin heavy chain gene rearrangement in these fractions since this provided an independent test for our ordering of the stages. We designed a polymerase chain reaction (PCR) amplification assay using pairs of oligonucleotide primers specific for regions 5' of J<sub>H</sub>1 and DFL6.1, sequences that are deleted upon rearrangement of D to J and V to D (Hardy et al. 1991). By amplifying these sequences together with an  $\alpha$ -actin gene segment (which serves to normalize for DNA loading and amplification efficiencies), we could measure levels of D to J and V to D-J rearrangement by quantitating decreases in the intensities of the corresponding fragments (which are resolvable on an agarose gel). Using this procedure we

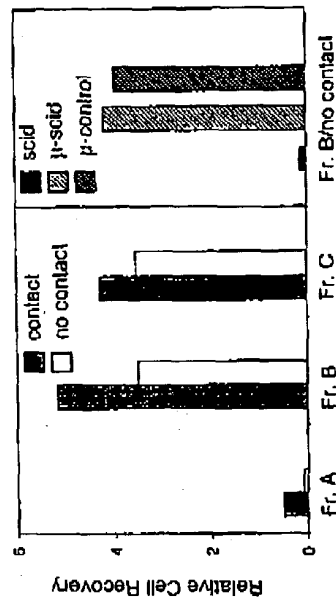
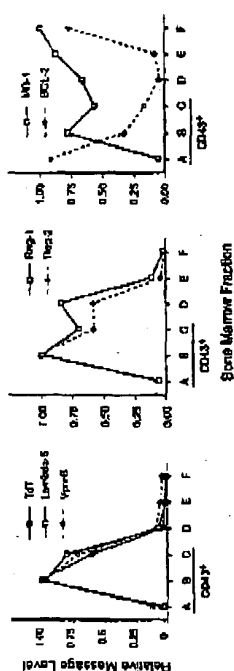


Figure 3. The IgH panel prescribes functional differences in B220<sup>+</sup>CD43<sup>+</sup> fractions in bone marrow revealed by culture directly on the (IL5T2 stromal layer (labelled "contact") or else in a diffusion chamber (labelled "no contact") suspended above the stromal layer. While Fr. A is absolutely contact-dependent for survival, Fr. C is essentially contact-independent. Cells ( $5 \times 10^4$ ) sorted directly into established cultures and analyzed 4 days later. The right panel presents a comparison of Fr. B cells isolated from SCID and p-transgenic SCID, supporting the model that productive Ig heavy chain rearrangement is necessary for the transition to contact-independent growth.

found that the Ig genes of Fr. A were in germline configuration, whereas those of Fr. B and C showed increasing D-J rearrangement, but no V-D-J (Table I). In contrast with these Pro B fractions, complete V-D-J rearrangements were seen in CD43<sup>+</sup> B lineage fractions (pre-B and B) in bone marrow.

#### Functional changes induced by immunoglobulin heavy chain expression

The alteration in growth dependence on contact-mediated signal(s) occurs at about the point that cells are completing heavy chain rearrangement. We have investigated this issue by comparing the growth of Pro B-cell fractions isolated from bone marrow of immunodeficient SCID mice with Pro B cells isolated from a SCID mouse bearing a rearranged immunoglobulin *H* transgene. The defect in SCID prevents the generation of significant numbers of productive Ig rearrangements and so Pro B cells in these mice initiate, but rarely complete, productive heavy chain recombination. We found that Fr. B cells from SCID could survive and even expand in conventional stromal cell culture, but that these cells could not grow (or even survive) in a diffusion chamber culture that allows soluble factors to reach the Pro B cells, but prevents direct contact with the stromal layer (Reidman-Triest et al. 1993). Interestingly, Pro B cells iso-



Bone Marrow Fraction

Figure 4. Gene expression in bone marrow as determined by RT-PCR. Message level is reported relative to the maximum value obtained in the analyses. Total RNA was extracted from  $10^6$  cells sorted directly into guanidinium thiocyanate lysis solution and then cDNA was synthesized using random hexamers and M-MLV reverse transcriptase. cDNA was amplified by PCR using the specific primers as described in Li et al. (1993). Aliquots were withdrawn at 22 and 26 cycle for separate analysis to ensure that amplification was within the linear range. To verify that equal amount of RNA were added in each PCR reaction, the "housekeeping gene"  $\beta$ -actin was also amplified. Samples were separated by 1.5% agarose gel electrophoresis, blotted and then hybridized with riboprobes prepared from cloned PCR products. Membranes were quantitated using a two-dimensional scintillation detector (Ambis, San Diego, CA).

ponents precede light chain rearrangement and the recombinase-activating genes are expressed in the three stages where heavy and light chain rearrangement are ongoing. Second, the similar pattern of expression seen with TdT, Lambda-5 and VpreB is consistent with the comparable promoter structure shared by these genes (lacking a TATA-box; Lo et al. 1991). Third, the absence of TdT-mediated N-regions from the junctions of light chains is simply accounted for by the precise restriction of TdT expression to stages where heavy chain, but not light chain, is occurring. Finally, the pattern of Bcl-2 is highly reminiscent of that seen with T-cell development in the thymus in that gene expression decreases strikingly at the immature receptor-positive stage, a point where selection and apoptosis is likely to be taking place.

In summary, it is possible to resolve six B-lineage fractions in the bone marrow of adult mice based on expression of CD45R1(B220/602), CD43(S7), HSA(10F1), BP-1, IgM and IgD. Three CD43(S7)<sup>+</sup> fractions represent very early B-lineage cells before or just at the stage of complete heavy chain rearrangement. The status of Ig rearrangement can be analyzed by both deletional and generational approaches, showing that the bulk of cells in Fr. B are D-J, but not V-DJ rearranged (Hardy et al. 1991, Ebelich et al. 1993, Li et al. 1993). Three CD43(S7)<sup>+</sup> fractions consist of Pre-B, immature B and mature B cells. Again, PCR rearrangement analysis shows that heavy chain rearrangement is complete by Fr. D, and that light chain rearrangement is largely restricted to the CD43<sup>+</sup> fractions (Hardy

lited from  $\mu$ -transgenic SCID bone marrow could proliferate in diffusion chamber culture, at about the level seen with control  $\mu$ -transgenic Pro B cells (Fig. 3). Thus it appears that expression of a productive Ig heavy chain signals the early B-lineage cell to alter its growth requirement, and proliferate strongly in response to soluble mediators. We found that growth comparable to diffusion chamber culture was obtained with recombinant IL-7 alone, which confirms the critical importance of this cytokine in B-cell development. A proliferative burst just after the stage of productive heavy chain rearrangement is reasonable in that such an expanded clone of cells would then initiate independent light chain rearrangements, generating a distinct set of immunoglobulin combining sites by combinatorial diversity.

#### Gene expression in bone marrow B-lineage subsets

A further test of this model of B-cell development has been the analysis of gene expression in these fractions. We utilized the polymerase chain reaction to amplify cDNA synthesized from total RNA isolated from these sorted bone marrow cell fractions in order to determine the level of expression of a set of B-lineage-associated genes (Li et al. 1993). The genes analyzed included MB-1, TdT, Lambda-5, Rag-1, BCL-2 and  $\beta$ -actin (which serves as a normalizing control for the amount of RNA and the efficiency of cDNA synthesis). As shown graphically in Fig. 4, this analysis reveals that: 1) MB-1, a molecule associated with immunoglobulin and critical for surface expression and signal transduction (Hombach et al. 1988, Lin & Justement 1992), is present from early stages of the B-lineage; 2) terminal deoxynucleotidyl transferase (TdT), an enzyme that mediates addition of nucleotides at the D-J and V-D junctions during heavy chain recombination (Landau et al. 1987), is expressed only during stages where such rearrangement is occurring. Pro B; 3) Lambda-5 and VpreB, that together form a light chain-like molecule that associates with heavy chain prior to light chain rearrangement (Kruszysny et al. 1990, Kudo et al. 1992), are largely restricted to stages prior to light chain rearrangement; 4) Rag-1 and Rag-2, "recombinase-activating genes" critically important in the process of Ig rearrangement at both heavy and light chain loci (Schaltz et al. 1989, Oettinger et al. 1990), are present in both Pro B and Pre-B cell stages; and 5) BCL-2, an oncogene important in maintaining the long-term survival of lymphoid cells (Vaux et al. 1988, McDonnell et al. 1989, Senftman et al. 1991, Strasser et al. 1991a, 1991b), is present at highest levels very early and then again late in the B-lineage pathway, but is found only at low levels in actively differentiating fractions.

One can draw several conclusions from these results. First, the expression of several of these genes confirms the proposed pathway of B-cell development: MB-1 is expressed from the earliest fractions, the surrogate light chain con-

99

## CD5 B CELLS: DEVELOPMENT AND SPECIFICITIES

Fr. A Fr. B Fr. C Fr. D Fr. E  
pre- Pro-B Pro-B small B cell  
Pro-B Pro-B Pro-B

## Cell Surface Phenotype

B220

S7

BP-1

HSA

## (g Rearrangement (%cells))

D-J <sub>H</sub>	30-60	100	100	100	100
V-D-J <sub>H</sub>	7183/052	51	10	16	20
J558	51	7	20	30	50
V-J <sub>H</sub>	51	14	35	50	95

## Gene Expression

Rag-1,  
Rag-2TdT,  
Lambda-5,  
Vpre-B

MB-1

bd-2

Figure 5. Diagram of bone marrow B-cell development showing cell surface phenotype, rearrangement status and gene expression in intermediate stages discussed in this review. The higher early rearrangement reported here was determined by positive PCR analysis (Li et al. 1993).

98 HARDY ET AL.

et al. 1991, Elhach et al. 1993, Li et al. 1993). Application of RT-PCR demonstrates tightly regulated gene expression that supports this model for B-cell differentiation and reveals further aspects of B-lineage associated gene regulation during lymphopoiesis in the bone marrow (Li et al. 1993). These results are presented diagrammatically in Fig. 5.

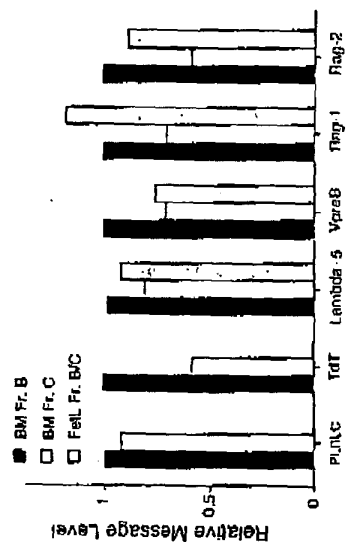
#### B-lineage differentiation in fetal liver: a developmental switch

We next sought to compare B lymphopoiesis in bone marrow and fetal liver. We reasoned that transfer of Pro B cell fractions might reveal restricted repopulations that would be obscured when using stem cells. That is, these more differentiated cells, if capable of giving rise to significant numbers of B cells in transfer assays, might already be committed to generating distinctive progeny. Transfer of Pro B cells sorted from bone marrow into sublethally irradiated immunodeficient SCID mice gave rise to B cells, but not T cells (Hardy & Hayakawa 1992). Furthermore, the limited numbers of cells used in these experiments ( $5 \times 10^4$ – $10^5$  per mouse) did not establish long-lasting B lymphopoiesis. Rather, after limited expansion, the cells differentiated into non-proliferating mature B cells which could be found in the spleen and peritoneal cavity.

Flow cytometry analysis of fetal liver revealed that essentially all B-lineage cells (defined as B220<sup>+</sup>) were CD43<sup>+</sup> at day 15/16 of gestation. That is, it appears that most B-lineage cells at this time are at the Pro B or earlier stage. Using the PCR rearrangement assay described above, such cells can be shown to possess extensive D-J, but not V-D-J, rearrangement consistent with their designation as "Pro B".

We then compared SCID recipients repopulated with such committed progenitors from bone marrow or fetal liver and found that distinctively different B cells were generated from Pro B cells present at these two developmental times. As early as 7–9 days following i.v. injection of these progenitors, B lymphocytes can be recognized in peripheral blood as B220<sup>+</sup> IgM<sup>+</sup> cells. All recipients of fetal progenitors show CD5 expression on at least some of these PBL B cells, whereas marrow progenitors show CD5 expression on at least some of these PBL B cells, whereas marrow progenitors. By 2 to 3 weeks after transfer, B cells are readily detectable in the spleen and peritoneal cavity (PerC). These spleen or PerC B cells repopulated from bone marrow progenitors appear "conventional" in phenotype (IgD<sup>+</sup> and CD5<sup>+</sup>) and show peak repopulation 2–3 weeks after transfer, decreasing thereafter. In contrast, B cells repopulated from fetal liver progenitors have an IgM<sup>+</sup>/IgD<sup>+</sup> phenotype and many express CD5. This population increases to maximal levels by 2 months after transfer, thereafter remaining constant. Representative flow cytometry analyses are shown in Fig. 6. These results prompt us to suggest that essentially all of the B-lineage cells early in development are committed to a distinct developmental pathway, eventually giving rise to a

## CDS B CELLS: DEVELOPMENT AND SPECIFICITIES 101



## Gene Analyzed

Figure 7. Gene expression in fetal liver compared to bone marrow as determined by RT-PCR. Both TdT and PLR1C are highly expressed in Pro B fractions from bone marrow (BM), but are absent from Pro B cells of fetal liver (Fet.). In contrast, Lambda-5, VpreB, Rag-1 and Rag-2 are expressed at comparable levels in both fractions. For fetal liver, Fr. B and C were not resolved. Analysis was performed as described in Fig. 4.

at the D-J and V-D junctions and decreased expression in fetal tissues had been reported previously using immunofluorescence techniques. However, we show essentially a complete absence from purified Pro B cells that are otherwise quite similar to the homologous fraction in adult bone marrow. PLR1C was recently described as differentially expressed in precursor lymphocytes from adult and fetal sources (Oltz et al. 1992) and our analysis confirms that this is also the case for purified Pro B cells.

In contrast to this differential expression of TdT and PLR1C, other genes critical to B-cell development such as Rag-1/2 and VpreB/Lambda-5 are comparably expressed. Thus, it appears that the homologous fractions from fetal liver and bone marrow share a number of features (ongoing D-J rearrangement, ability to grow on a support stromal layer and expression of genes such as Rag-1 and Lambda-5), but also exhibit differences in expression of other genes and give rise to phenotypically distinct sets of mature B cells in identical SCID recipients. Absence or decreased levels of TdT will reduce CDR3 diversity and conceivably could result in increased homology-mediated recombination (favoring certain V-D or D-J combinations). This could lead to a difference in the repertoire of antigen specificities expressed by the fetal-generated B-cell population compared to adult B cells. The function of PLR1C is unknown at present, but homology to a structural gene (myosin light chain) suggests a role in the cytoskeleton so

## HARDY ET AL.

100

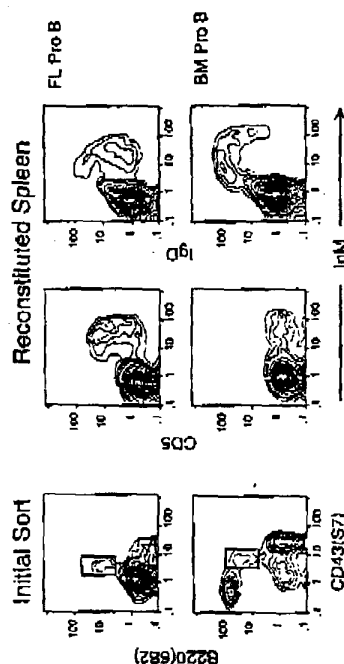


Figure 6. Distinct B-cell phenotypes reconstituted 3 weeks after transfer of Pro B cell fractions from fetal liver and adult bone marrow. Pro B cell fractions (1-2% of total cells) were isolated from day-16 fetal liver and bone marrow of adult (>3 months) mice by cell sorting, then injected i.v. into SCID mice ( $1 \times 10^6$  animals) irradiated 100R the previous day. Mice were sacrificed 3 weeks later and cells from spleen were stained and analyzed as indicated. Bone marrow and thymus showed essentially no engraftment.

phenotypically resolvable population of B cells, many of which are CDS<sup>+</sup> (Hardy & Hayakawa 1991).

This result contrasts with our earlier expectation that two distinct pathways would be present in fetal liver and that one would diminish with age. Instead we see evidence of a "developmental switch" in B lymphopoiesis, from a fetal type early on to an adult type in bone marrow by 2-3 months of age.

## Gene expression in fetal liver B-lineage fractions

If the destiny of B-lineage cells in identical microenvironments differs depending on the Pro B cell source, then this implies that gene expression at the Pro B cell stage should already be different. Therefore, we have begun to use the RT-PCR assay described above for bone marrow B-lineage fractions to analyze fetal liver in order to understand the mechanism for generation of the phenotypically distinct mature B-cell populations that we observed in the SCID transfer experiments. Our initial results (Fig. 7) are quite striking: whereas many B-lineage genes are expressed in fetal liver at levels comparable to the Pro B cell stage in bone marrow, two genes analyzed (TdT and the precursor lymphocyte regulated myosin-like light chain, PLR1C) are not. Both of these genes are highly expressed in Fr. B of bone marrow but absent from fetal liver. TdT mediates N-nucleotide addition

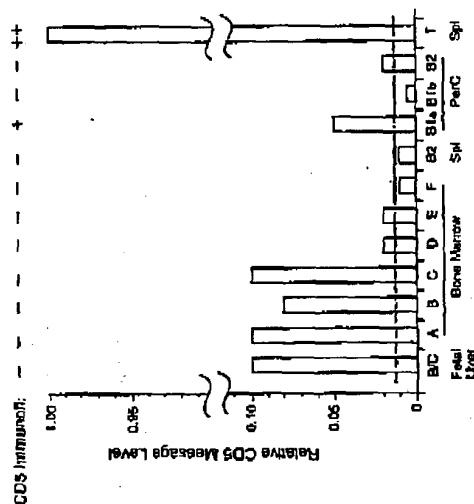


Figure 8. CDS RT-PCR expression analysis showing discordant CDS message and immunofluorescence cell surface expression. RT-PCR analysis was performed as described in Fig. 4. For peripheral cells, B1a refers to  $IgM^{+}IgD^{+}CDS^{+}$  cells, B1b means  $IgM^{+}IgD^{+}CDS^{-}$  cells and B2 is  $IgM^{+}IgD^{+}CDS^{-}$ . All values are given relative to CDS expression in spleen T cells (=1.00). Immunofluorescence analysis of CDS presents relative surface expression detected by the  $\lambda 3-7$  m1 anti-CDS antibody. The grey line indicates background level of detection by RT-PCR.

that differential expression might mean that the coupling of surface molecules to intracellular proteins might differ. It is reasonable to predict that expression of TdT and PLR1C will not be the only differences between fetal and adult B lymphopoiesis responsible for the distinct mature B-cell phenotypes.

#### Expression of CDS in B-cell development

Expression of CDS as determined by surface immunofluorescence on the cell sorter serves to mark a functionally distinct subset of B cells so it is interesting to determine when CDS is first detectable during B-cell development. By surface immunofluorescence, CDS is undetectable on B-lineage surface  $Ig^{-}$  cells in both fetal liver and bone marrow. Furthermore, culture of fetal liver Pro B generates  $IgM^{+}$  cells, and a fraction of these begin to express CDS, whereas the  $IgM^{+}$  cells in these cultures are  $CDS^{-}$ . Thus, CDS expression would appear to be a relatively late-appearing marker during B-cell differentiation, in contrast to its expression early in T-cell development.

We have also analyzed CDS message expression using an RT-PCR assay. The results, shown in Figure 8, reveal one difference from the surface immunofluorescence analysis: CDS message is detectable at the earliest stages of B-cell differentiation (Pro B and Pre-Pro B) in both bone marrow and fetal liver, but decreases strikingly during bone marrow B-cell differentiation. Similar analysis of peripheral B-cell subsets shows good correspondence between message levels and surface expression assessed by flow cytometry. We do not as yet understand the significance of the discordant CDS expression early in B-cell development, but speculate that it could be cytoplasmic-restricted at low levels that would be difficult to demonstrate by standard protein analysis techniques.

#### BIASED SPECIFICITIES AND $Ig V_H$ GENE USAGE IN $CDS^{+}$ B CELLS

One of the most distinctive features of the  $CDS^{+}$  B-cell subset is its association with certain types of autoantibody. Thus,  $CDS^{+}$  B cells are uniquely elevated in the autoimmune NZB mouse strain (Hayakawa et al. 1983) and most of the cells in severely autoimmune, nonhealed strain are  $CDS^{+}$  (Skinner et al. 1986). In addition, "natural" autoantibodies such as antibody to bromelain-treated mouse red blood cells (BrMRBC) and anti-thymocyte autoantibody (ATA) were shown early on to be distinctively enriched in  $CDS^{+}$  B cells, even in normal mouse strains (Hayakawa et al. 1984). In this review we describe our work on antibody to thymocytes and to BrMRBC.

#### The anti-bromelain-treated red blood cell specificity

A difference in the antibody repertoire between these two B-cell subsets ( $CDS^{+}$   $IgD^{+}$  and  $CDS^{+}$   $IgD^{-}$ ) in adult mice was first suggested by exclusive anti-

BrMRBC (bromelain-treated mouse red blood cells) autoantibody secretion from  $CDS^{+}$  B cells (Hayakawa et al. 1984). This was demonstrated by using both BALB/c and SM/J mice. Results showed that depletion of  $CDS^{+}$  B cells from the spleen specifically eliminated production of this autoantibody and conversely, that purified  $CDS^{+}$  B cells uniquely exhibited anti-BrMRBC secretion elicited by LPS (Hayakawa et al. 1984; Hardy et al. 1989). The screening of hybridomas generated from sorted  $CDS^{+}$  B cells and  $CDS^{+}$   $IgD^{+}$  B cells confirmed that the  $CDS^{+}$  B cell population was indeed enriched for cells with this specificity (Fig. 9). As mentioned above, by comparing hybridomas generated from splenic  $CDS^{+}$  B cells and  $CDS^{+}$  B cells sorted from SM/J strain mice we found that anti-BrMRBC-producing hybridoma clones comprised 1–2% of the hybridomas from  $CDS^{+}$  B cells, but only 0.03% in hybridomas made from  $CDS^{+}$   $IgD^{+}$  B cells (Hardy et al. 1989).

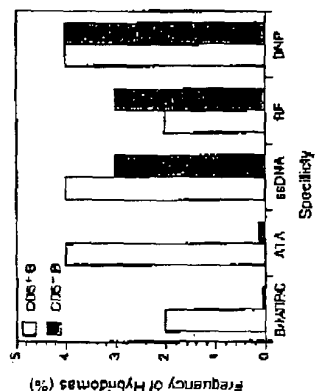


Figure 9. Anti-Br MHC and ATA reactivity is enriched in CD5 B-cell derived hybridomas, whereas other classes of autoreactivity such as tDNA or rheumatoid factor (RF) are not.

*N<sub>1</sub>* gene usage in anti-B<sub>1</sub>A RBC hybridomas: T<sub>H</sub>1 and T<sub>H</sub>2

A gene from the  $V_{H}11$  family is predominantly utilized in anti-BrMRBC B cells in NZB and C3H mice (Reininger et al. 1987). We found that this was also the case for SM/J mice. Sequencing the heavy chain V-genes of two anti-BrMRBC hybridomas (Lardy et al. 1989) from SM/J showed repeated usage of this gene in association with a member of the  $V_{H}9$  family. We cloned the  $V_{H}11$  gene and found, using Southern analysis, that 14/16 of our anti-BrMRBC hybridomas expressed  $V_{H}11$  rearrangements. Using the cloned gene we showed that the highly homologous  $V_{H}$  genes were very few in number (2-3) so that  $V_{H}11$  defines one of the smallest  $V_{H}$  families. Also, analysis of DNA from mouse lines that had undergone recombination within the  $V_{H}$  locus showed that both of these  $V_{H}11$  genes mapped upstream of the S107 cluster. This means that  $V_{H}11$  is not among the genes located proximal to D, genes that have been suggested to be over-represented early in development. The small fraction of anti-BrMRBC hybridomas that did not utilize  $V_{H}11$  (2/16) had rearranged another novel  $V_{H}$  gene, one that did not utilize  $V_{H}11$  (Punnett et al. 1989). This family, with only one member, was originally described in a CD5<sup>+</sup> B lymphoma (Punnett et al. 1988) and has also been seen in hybridomas generated from B cells washed out of the peritoneal cavity, a rich source of CD5<sup>+</sup> B cells (Mercedino et al. 1989).

*U1* T-antigen in normal B cells

This enrichment of V $\beta$ 11-encoded anti-B $\alpha$ MRBC specificity in the CD5<sup>+</sup>  $\beta$ -cell subset was also demonstrated with normal cells of BALB/c mice by analysis of

**CD5<sup>+</sup> B-cell DNA rearrangement.** Using PCR, we found enriched  $V_H11/12/JH1$  rearrangement in Ly-1<sup>+</sup> B cells from both spleen and peritoneal cavity as compared to either bone marrow pre-B cells or adult B cells ( $\lg D^{+}/CD5^{-}$ ) from either spleen or PerC (Carmack et al., 1998). We designed oligonucleotide primers homologous to the  $V_H11$  leader sequence and  $J_H1$  (the rearrangement seen in all hybridomas analyzed) and carried out amplification together with a set of primers that amplified another region of DNA upstream of C $\mu$  that served to normalize the  $V_H11$  signal (see Fig. 10). Our data (Table II) clearly demonstrated a difference in repertoire (Fig. 10). CD5<sup>+</sup> B-cell subsets, even when located in the same tissue. They also showed that  $V_H11$  does rearrange in bone marrow (and its progeny, CD5<sup>+</sup> cells of spleen) at about the frequency that would be expected for a germ-line family. This one explanation for the over-representation of  $V_H11/JH1$  in CD5<sup>+</sup> B cells, that somehow the promoter/enhancer structure restricts its rearrangement in fetal B cells, appears unlikely. Together with the finding that  $V_H11$  (and  $V_H12$ ) are not located proximal to D, these results suggest that the initial V gene usage of CD5<sup>+</sup> B cells may not be strikingly different from that of bone marrow-derived B cells. Rather, it appears likely that is selection



Figure 1a. Diagram for  $V_{H1}$  PCR used to generate the data shown in Table II. A  $V_{H1}$  primer combination amplified  $V_{H1}$  rearranged DNA and simultaneously a separate pair of primers amplified a DNA fragment present in all IgM<sup>+</sup> B cells. This normalizing fragment was chosen because it was also present on the cloned  $V_{H1}$  sequence.

TABLE II  
Field reorganization in B-cell subsets

Cell type	Frequency of cells (%)	V <sub>H</sub> 1-J <sub>H</sub> 1	
		(%)	SE
BAL Pro-B	8	0.7	0.1
BM B	10	0.8	0.3
Spl IgD <sup>+</sup> B	50	0.9	0.1
Spl CD5 <sup>+</sup> B	2	9.0	1.3
PerC IgD <sup>+</sup> B	5	1.0	0.1
PerC CD5 <sup>+</sup> B	26	10.0	1.1
Spl T cells	30	0.2	0.1

operating on the CD5<sup>+</sup> B-cell population that results in the extreme biases that are found with certain specificities, particularly autoreactive ones.

Passive selection in CD5<sup>+</sup> B cells

The initial repertoire of CD5<sup>+</sup> B cells generated during the fetal stage has not been examined as yet. However, a change in this repertoire with ontogeny has been suggested by an increase in the frequency of anti-DrMRBC-specific B cells within the CD5<sup>+</sup> B-cell subset. Mercolino et al. found that anti-DrMRBC-specific B cells, detected as TrC (phosphatidyl choline)-binding cells, increased with age during normal ontogeny (Mercolino et al. 1988). We observed that this was also the case during the B-cell reconstitution period for recipients of fetal B-cell progenitors (Hardy & Hayakawa 1991). In contrast, mice reconstituted with newborn marrow did not develop a population of DrMRBC-specific B cells, even 1 year after stem cell transfer.

The enrichment of anti-Bm1RBC B cells in CD5<sup>+</sup> B cells is more evident when analyzing cells in the peritoneal cavity where CD5<sup>+</sup> B cells preferentially reside (Hayakawa et al. 1986). More than 30% of IgM<sup>+</sup> B cells present in the PerC are anti-Bm1RBC B cells. Ten percent of CD5<sup>+</sup> B cells in the PerC of adult BALB/c mice are anti-Bm1RBC B cells, detectable as antigen (PIC)-binding B cells (Mercolino et al. 1983). We found that such B cells are also specifically detected as B cells labeled by anti-Bm1RBC antibody itself (Carmack et al. 1990). Cells capable of anti-Bm1RBC secretion and bearing V<sub>H</sub>11 gene rearrangement are uniquely labeled by anti-Bm1RBC antibodies, but not by antibodies with other specificities. This strongly suggests a constant occupancy of the anti-Bm1RBC Ig receptor by a multivalent antigen which would allow specific "sandwich" staining. Thus one likely reason for preferential selection and maintenance of CD5<sup>+</sup> B cells is the availability of antigen. Certain autoantigens may serve as such antigens if corresponding autoantibody specificity affinity can be encoded by germline genes.

Nearly identical  $V_{H1}$  gene sequences have been found with anti-BmBRBC B<sub>2</sub> hybridomas established from a pool of mice and from several different strains. This suggests that the specificity is germline gene-encoded with no somatic mutation. Further, the increase of anti-BmBRBC specificity in the CD5<sup>+</sup> B-cell subset was not simply due to clonal proliferation of one or a few rearrangements. This was suggested by the presence of anti-BmBRBC hybridomas using different J<sub>H</sub> (Larsley et al. 1989).

estimates of the effect of the intervention on the outcome.

The presence of numerous unrelated clones in the anti- $\alpha$ -MRBC B cell fraction was confirmed by cloning and sequencing of V $\alpha$ 1 Ig J $\alpha$  rearranged DNA by

PCR amplification from CD5<sup>+</sup> B cells of a single DALY/c mouse which revealed many distinct CDR3 regions. These sequences (Fig. 1) showed a relatively diverse set of rearrangements within the constraints already noted in sequence analysis of anti-B $\mu$ MRBC hybridomas: relatively short CDR3 regions and little N-N-addition. Half of the clones sequenced have no obvious N-addition, while many of the rest have only short N-sequences at the D-J junctions. This is consistent with the low levels of TdT expected to be present during rearrangement in fetal Pro B cells. However, about a quarter of the sequences show significant N-N-addition (4 or more bases), suggesting that the precursors of at least some CD5<sup>+</sup> B cells derive from Pro B cells expressing higher levels of TdT. Perhaps some cells differentiating along the proposed "fetal pathway" begin to express TdT toward the end of this process (at 1-2 weeks after birth) or else strong selection for  $\mu$ MRBC-binding cell enriches even rare cells containing some N-addition. One clear point is that 25% of V $\mu$ 11 rearrangements in CD5<sup>+</sup> B cells contain readily detectable N-addition and so the presence such sequences does not block a cell from entering this population. This analysis also shows common clones present in CD5<sup>+</sup> B cells in both spleen and peritoneal cavity suggesting a common precursor or else trafficking between the two compartments.

Translation of the nucleotide sequences (Fig. 12) shows evidence of a preference for tyrosines and serines in the  $V_{H1-1}J_{H1}$  CDR3 region. This may arise partly

V <sub>H</sub> 11	D	$\delta_{H1}$	No. of Clones SPN	Diversity
TCACGTTAC		TCACGTTAC	3	572
TCATGTTAC		TCATGTTAC	1	573
TCATGTTAC		TCATGTTAC	6	574
TCATGTTAC	GGTA	TCATGTTAC	1	575
TCATGTTAC	TCGCG	TCATGTTAC	2	576
TCATGTTAC	TCGCG	TCATGTTAC	1	577
TCATGTTAC	TCGCG	TCATGTTAC	2	578
TCATGTTAC	TCGCG	TCATGTTAC	1	579
TCATGTTAC	TCGCG	TCATGTTAC	1	580
TCATGTTAC	TCGCG	TCATGTTAC	2	581
TCATGTTAC	TCGCG	TCATGTTAC	1	582
TCATGTTAC	TCGCG	TCATGTTAC	1	583
TCATGTTAC	TCGCG	TCATGTTAC	1	584
TCATGTTAC	TCGCG	TCATGTTAC	1	585
TCATGTTAC	TCGCG	TCATGTTAC	1	586
TCATGTTAC	TCGCG	TCATGTTAC	1	587
TCATGTTAC	TCGCG	TCATGTTAC	1	588
TCATGTTAC	TCGCG	TCATGTTAC	1	589
TCATGTTAC	TCGCG	TCATGTTAC	1	590
TCATGTTAC	TCGCG	TCATGTTAC	1	591
TCATGTTAC	TCGCG	TCATGTTAC	1	592
TCATGTTAC	TCGCG	TCATGTTAC	1	593
TCATGTTAC	TCGCG	TCATGTTAC	1	594
TCATGTTAC	TCGCG	TCATGTTAC	1	595
TCATGTTAC	TCGCG	TCATGTTAC	1	596
TCATGTTAC	TCGCG	TCATGTTAC	1	597
TCATGTTAC	TCGCG	TCATGTTAC	1	598
TCATGTTAC	TCGCG	TCATGTTAC	1	599
TCATGTTAC	TCGCG	TCATGTTAC	1	600
TCATGTTAC	TCGCG	TCATGTTAC	1	601
TCATGTTAC	TCGCG	TCATGTTAC	1	602
TCATGTTAC	TCGCG	TCATGTTAC	1	603
TCATGTTAC	TCGCG	TCATGTTAC	1	604
TCATGTTAC	TCGCG	TCATGTTAC	1	605
TCATGTTAC	TCGCG	TCATGTTAC	1	606
TCATGTTAC	TCGCG	TCATGTTAC	1	607
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TCATGTTAC	TCGCG	TCATGTTAC	1	614
TCATGTTAC	TCGCG	TCATGTTAC	1	615
TCATGTTAC	TCGCG	TCATGTTAC	1	616
TCATGTTAC	TCGCG	TCATGTTAC	1	617
TCATGTTAC	TCGCG	TCATGTTAC	1	618
TCATGTTAC	TCGCG	TCATGTTAC	1	619
TCATGTTAC	TCGCG	TCATGTTAC	1	620
TCATGTTAC	TCGCG	TCATGTTAC	1	621
TCATGTTAC	TCGCG	TCATGTTAC	1	622
TCATGTTAC	TCGCG	TCATGTTAC	1	623
TCATGTTAC	TCGCG	TCATGTTAC	1	624
TCATGTTAC	TCGCG	TCATGTTAC	1	625
TCATGTTAC	TCGCG	TCATGTTAC	1	626
TCATGTTAC	TCGCG	TCATGTTAC	1	627
TCATGTTAC	TCGCG	TCATGTTAC	1	628
TCATGTTAC	TCGCG	TCATGTTAC	1	629
TCATGTTAC	TCGCG	TCATGTTAC	1	630
TCATGTTAC	TCGCG	TCATGTTAC	1	631
TCATGTTAC	TCGCG	TCATGTTAC	1	632
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TCATGTTAC	TCGCG	TCATGTTAC	1	659
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TCATGTTAC	TCGCG	TCATGTTAC	1	663
TCATGTTAC	TCGCG	TCATGTTAC	1	664
TCATGTTAC	TCGCG	TCATGTTAC	1	665
TCATGTTAC	TCGCG	TCATGTTAC	1	666
TCATGTTAC	TCGCG	TCATGTTAC	1	667
TCATGTTAC	TCGCG	TCATGTTAC	1	668
TCATGTTAC	TCGCG	TCATGTTAC	1	669
TCATGTTAC	TCGCG	TCATGTTAC	1	670
TCATGTTAC	TCGCG	TCATGTTAC	1	671
TCATGTTAC	TCGCG	TCATGTTAC	1	672
TCATGTTAC	TCGCG	TCATGTTAC	1	673
TCATGTTAC	TCGCG	TCATGTTAC	1	674
TCATGTTAC	TCGCG	TCATGTTAC	1	675
TCATGTTAC	TCGCG	TCATGTTAC	1	676
TCATGTTAC	TCGCG	TCATGTTAC	1	677
TCATGTTAC	TCGCG	TCATGTTAC	1	678
TCATGTTAC	TCGCG	TCATGTTAC	1	679
TCATGTTAC	TCGCG	TCATGTTAC	1	680
TCATGTTAC	TCGCG	TCATGTTAC	1	681
TCATGTTAC	TCGCG	TCATGTTAC	1	682
TCATGTTAC	TCGCG	TCATGTTAC	1	683
TCATGTTAC	TCGCG	TCATGTTAC	1	684
TCATGTTAC	TCGCG	TCATGTTAC	1	685
TCATGTTAC	TCGCG	TCATGTTAC	1	686
TCATGTTAC	TCGCG	TCATGTTAC	1	687
TCATGTTAC	TCGCG	TCATGTTAC	1	688
TCATGTTAC	TCGCG	TCATGTTAC	1	689
TCATGTTAC	TCGCG	TCATGTTAC	1	690
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TCATGTTAC	TCGCG	TCATGTTAC	1	695
TCATGTTAC	TCGCG	TCATGTTAC	1	696
TCATGTTAC	TCGCG	TCATGTTAC	1	697
TCATGTTAC	TCGCG	TCATGTTAC	1	698
TCATGTTAC	TCGCG	TCATGTTAC	1	699
TCATGTTAC	TCGCG	TCATGTTAC	1	700

**Figure 11. Functional diversity of  $V_{H}1.1/J_{H}1$  rearrangements in CD5<sup>+</sup> B cells from a single patient.** Nucleotide sequences obtained after PCR amplification of  $V_{H}1.1/J_{H}1$  rearrangements from sorted CD5<sup>+</sup> B cells of spleen and peritoneal cavity. DNA prepared from sorted samples was amplified using primers that contained restriction sites to facilitate cloning. Amplified DNA was then cut with appropriate restriction enzymes, cloned and the clones sequenced. Non-germline nucleotides are indicated between the junctions in the above cases. The number of instances of each sequence in the spleen and peritoneal cavity samples is indicated.



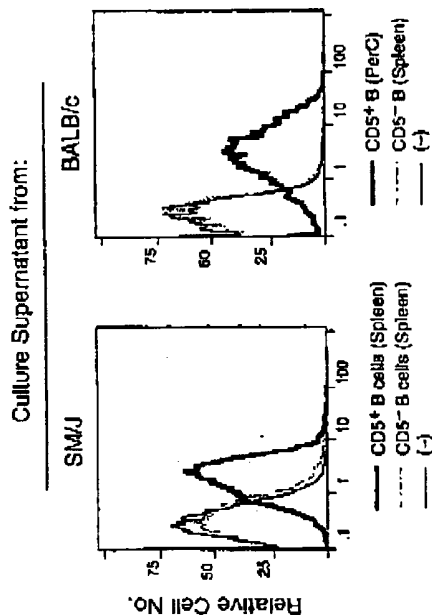


Figure 14. Anti-dynectin autoantibody activity (ATA) in supernatants of LPS-stimulated sorted spleen or peritoneal washout cell fractions from SM/J and BALB/c mice. CD5<sup>+</sup> B cells or CD5<sup>+</sup> B cell fractions were isolated by cell sorting and cultured. Only CD5<sup>+</sup> B cell fractions produced ATA. "—" indicates the no-tissue culture background.

ATA found in this mouse strain.

**ATA specificities: carbohydrate determinants dependent on Thy-1 expression**

ATA from CD5<sup>+</sup> B cells was characterized further by generating hybridomas. Hybridomas made from CD5<sup>+</sup> B cells and conventional CD5<sup>+</sup> (IgD<sup>+</sup>) B cells

competition with *Eco* RI. Digested DNA was electrophoresed in a 1% low melting agarose gel and the  $V_{H1}$  germline bands around 8.4 kb were cut out and the DNA isolated and subcloned into  $\lambda$ -Zap II (Stratagene, La Jolla, CA) and the partial library screened with a  $V_{H1}$  probe (Hardy et al. 1983). Positives were identified and recovered.  $\lambda$  Zap II was then induced with helper phage to automatically excise the pBluescript plasmid vector contained within, yielding a plasmid vector. Positive plasmids were recovered and purified by spinplasmid was digested with EcoRI/mung bean nuclease (Stratagene) to produce a series of deletions of the germline  $V_{H1}$  gene. Nested deletions were then sequenced using the universal primer contained in the plasmid. Contig fragment sequences were assembled in GCG. Sequence data of two or more overlapping clones are shown as upper case letters indicate only one clone with readable sequence at that position).

HARDY ET AL.

211

[illegible]

**Figure 1.** Nucleotide sequence of 2.4 kb of the  $V_{H1}$  germline gene. Sequence starts from the EcoRI site (GAATTC). Amino acid translation of coding region is shown above the nucleotide sequence in brackets and these are numbered according to Kabat. Unclassified region (indicated by asterisks) is identical to the sequence in the  $V_{H1}$  gene of the  $\mu$  chain of the IgM antibody. High molecular weight DNA was isolated from the liver of SM1 mice by conventional methods and digested to

## CD5 B CELLS: DEVELOPMENT AND SPECIFICITIES 113

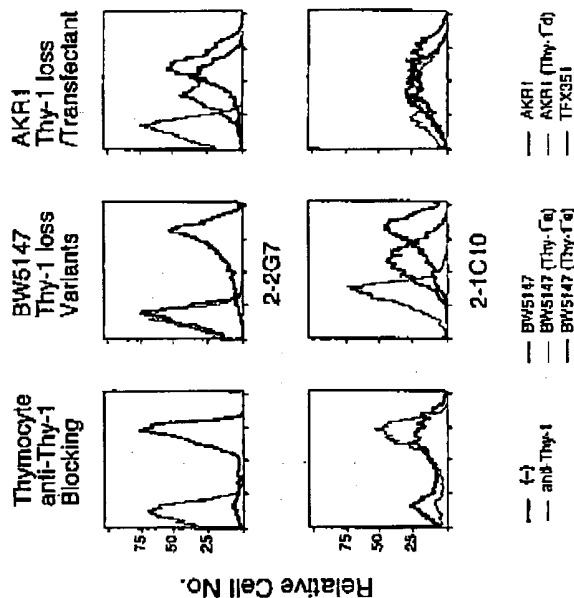


Figure 15. Examples of ATA hybridomas recognizing two distinct epitopes. One (2-2G7) is completely blocked by anti-Thy-1 antibody, whereas the other (2-1C10) is unaffected. 2-2G7 staining is also lost on BW5147 Thy-1 variant lines whereas 2-1C10 staining is not. Finally, transfection of a Thy-1 gene into an AKR1 Thy-1 variant line (TFX351) restores 2-2G7 staining. All these results suggest that 2-2G7 is reacting with a determinant on (or very closely associated with) the Thy-1 glycoprotein.

identical  $V_H$  genes, one utilizing 3609, another with a J558 member and a third with a different J558 gene. Two hybridomas derived from a clonal expansion since they had identical V-D-J sequences. However, we also found repetitive usage of an identical  $V_H$  gene not due to clonal proliferation. The four 3609 hybridomas are unrelated, as shown by D and J $\alpha$  differences. Another pair used identical  $V_H$  and  $J_H$  genes, but were unrelated, as distinguished by their D genes. In conclusion, repetitive  $V_H$  gene usage in ATA hybridomas is due both to clonal expansion and to independent incidence and is consistent with antigenic selection. It is worth noting that repetitive usage of particular Ig V genes by CD5<sup>+</sup> B cells has been reported previously by others (Forster et al. 1988, Stall et al. 1988, Tarleton et al. 1988).

## 112 HARDY ET AL.

sorted from a pool of SM/3 mouse spleen were plated in limiting dilution to assess the frequency of clones/well as described previously (Hayakawa et al. 1988). As described above (Fig. 9), the initial screening of 576 IgM-secreting wells from both CD5<sup>+</sup> B cells and CD5<sup>+</sup> B cell fusions showed that the frequency of clones with anti-thymocyte binding reactivity was at least 10 times higher in CD5<sup>+</sup> B cells, consistent with results of the ATA-secreting assay described above. Further analysis of such hybridoma clones revealed several distinct ATA binding profiles to different cell types (Hayakawa et al. 1990). Most monoclonal IgM ATA from CD5<sup>+</sup> B cells selected for thymocyte binding activity showed specific binding to T-lineage cells (among hematopoietic cells tested). However, their specificities appeared diverse, exhibiting differential reactivity to thymocytes at various maturational stages, variable binding to peripheral T cells (CD4<sup>+</sup> and/or CD8<sup>+</sup>), and different degrees of species crossreactivity (mouse/rat). Most reacted with thymocytes from all strains of laboratory mice tested including SM/J, the strain from which the hybridomas were derived.

The reactivity of 11 of these autoantibodies was sensitive to prior treatment of target cells with sodium metaperiodate which preferentially oxidizes terminal sialic acid groups, implicating carbohydrate as an important constituent of the antigenic determinant (Hayakawa et al. 1990). Further, as Thy-1 is a major glycoprotein of the thymus, we investigated the ability of anti-Thy-1 antibody to block ATA binding. The binding of eight ATA antibodies was nearly completely blocked by prior or simultaneous incubation of thymocytes with anti-Thy-1 antibody, while the binding of two other ATA antibodies was partially blocked (Fig. 15). Blocking by anti-Thy-1.1 or anti-Thy-1.2 antibodies was specific to the appropriate Thy-1 allele and other antibodies (anti-CD45, anti-CD3) which react with determinants present on the majority of thymocytes did not block. This result suggests that a determinant detected by many of these antibodies either resides on the Thy-1 glycoprotein or on a molecule that is present in close proximity to Thy-1. Indeed, reactivity was lost by a cell line selected for absence of cell surface Thy-1 expression and restored upon transfection of this line with the Thy-1 gene. Several other ATA, such as 2-1C10, were found to react with glycolipids unrelated to Thy-1.

#### $V_H$ gene usage in ATA hybridomas

The differential staining profiles, reactivities and Thy-1 inhibition of these ATA hybridomas suggested that their antigenic determinants could be quite diverse. This was confirmed by sequencing the immunoglobulin V genes of these hybridomas (Hayakawa et al. 1990). We found that 14 Ly-1 B-derived ATA hybridomas utilized  $V_H$  genes belonging to four distinct families Q558, 3609, Q52, Vgam3.8). Notably, our data revealed that fine specificities definable by our assays largely correlated with usage of particular  $V_H$  genes. Three groups of ATA used nearly

## CONCLUDING REMARKS

Our current understanding of CD5<sup>+</sup> B cells is that they are the progeny of a "distinct lineage" of B-cell differentiation on the basis of developmental origin. As shown by differences in the levels of CD5 and IgD, we presume that the control of expression of several genes will differ significantly between these B-cell lineages. Our data also suggest that Ly-1 B cells, generated early in ontogeny, are selected to maintain their progeny by the presence of "natural" antigens and without a requirement for T-cell help. In contrast, B cells generated from adult bone marrow are not selected in this manner, even though they are capable of rearranging the same genes. This model of B-cell development is diagrammatically depicted in Fig. 16. The molecular basis of such physiological distinctions between fetal and adult B cells remains to be elucidated. Nevertheless, our results find

## A Developmental Switch in B Lymphopoiesis

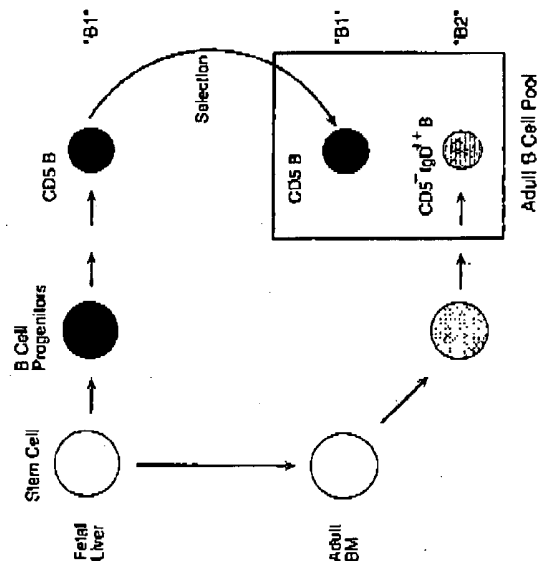


Figure 16. Proposed model of B-cell differentiation at distinct developmental timings yielding CD5<sup>+</sup> B cells (referred to as "B1") fully and conventionally B cells (CD5<sup>+</sup> IgD<sup>+</sup> B, referred to as "B2") in the adult.

others) lead us to consider that fetal B cells may serve as an inherited immune system to natural (self-) antigens. Within this population, quantitative increases of B cells with useful specificities could provide the mechanism for a type of "memory" maintained through life.

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## CD5 B CELLS: DEVELOPMENT AND SPECIFICITIES 117

## 116 HARDY ET AL.

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## Sex Hormones as Negative Regulators of Lymphopoiesis

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### SUMMARY

B lymphocytes, together with cells of seven other lineages, are made in large numbers from precursors in the bone marrow. Using cell culture models and recombinant proteins, progress has been rapid in identifying cytokines which could potentially regulate the proliferation, differentiation and migration of B-cell precursors. However, we still know little about molecular mechanisms which are important for maintaining steady-state conditions *in vivo*. B lymphopoiesis is severely diminished during pregnancy in normal mice and this provided a clue that sex hormones might be important negative regulators. Administration of IL-7 responding cells and their progeny in bone marrow, preferentially suppressed these observations in the thymus, which transiently involutes during pregnancy, and also atrophies following estrogen treatment. The actual mechanism(s) through which sex steroids influence lymphopoiesis remain unclear, but cell culture experiments should be informative about potential interactions between hormones, the bone marrow microenvironment, and lymphocyte precursors. These findings raise a number of other important issues. For example, we need to learn if sex steroids are produced and/or concentrated locally within the marrow; if human lymphopoiesis is sensitive to these hormones, and if production of lymphocytes can be augmented in aging and in immunodeficiency by hormone manipulation.

### INTRODUCTION

Blood cell formation initiates during embryonic development and eventually becomes established within the bone marrow. Thereafter, the production of these

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